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STUDIES ON OXIDATION-REDUCTION

XI. POTENTIOMETRIC AND SPECTROPHOTOMETRIC STUDIES OF BINDSCHEDLER'S GREEN AND TOLUYLENE BLUE

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Introduction

Data which may become of considerable importance to cytology and to general physiology are being obtained by injecting indophenols into single cells. A recent review of this subject is given by J. Needham and D. M. Needham (1926). An interpretation of the results depends upon the use of the equilibrium potentials of each indophenol system the data for which are to be found in the third, fifth, sixth, and seventh papers of this series.

It would be desirable to repeat the microinjection experiments with oxidation-reduction indicators different in type, but having that range of potential which has made the indophenols so useful. An obvious first step in this direction is to substitute indamines for indophenols. These two series of compounds have the same general structure and enough is now known of the effect of general structure upon oxidation-reduction potentials to suggest that the similarity will be revealed in a similarity of potential-range. The structural difference between the two sets of compounds is essentially the substitution of basic groups for acidic groups. Perhaps this is undesirable for biological purposes; yet methylene blue, which contains a very strong and also a very weak basic group, has yielded valuable results in the study of biological oxidation-reduction. It remains to be seen whether or not basic groups in themselves introduce difficulties in the biochemical applications of oxidation-reduction indicators; and for the study of this problem it will be desirable to have data on at least two indamines.

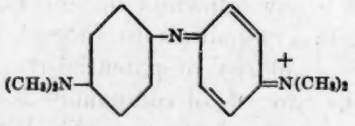
Should these substances prove to be useful, they have a certain decided advantage over indophenols. The dissociation constants of indamines usually are such that high color of the oxidant is retained throughout a considerable range of pH centered at neutrality. It will be recalled that the simple indophenols yield highly colored solutions in *alkaline* solutions *only*, and that it was necessary to halogenate them in order to raise the dissociation constants sufficiently to retain high color at neutrality.

Aside from these matters of immediate importance, data on indamines should be of value in several other directions. Compounds of indamine type, of which Bindschedler's green is representative, and compounds of type intermediate between that of an indamine

and that of an indophenol appear frequently as the colored products in numerous color tests. Most noteworthy is the case of the so-called indophenol reaction which is used empirically to differentiate leucocytes and which, when applied to the differentiation of events within the living cell, has furnished the uncertain basis of far-reaching conclusions. As a preliminary to a more exact interpretation of the common indophenol reaction, it will be necessary to have at hand some data on the equilibrium potentials of the product formed, and, in turn, a preliminary to this is the elucidation of at least one indamine system.

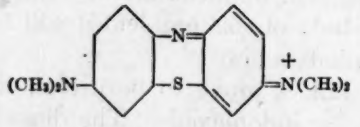
There are described in this paper the equilibrium potentials of mixtures of Bindschedler's green and its leuco product and of mixtures of toluylene blue and its leuco product. Incidentally, certain spectrophotometric measurements were made with each oxidant, and certain hydrogen electrode measurements were made with each reductant.

Bindschedler's green, which was first made by Bindschedler (1880), was subsequently characterized by Nietzki (1883) and by Bindschedler (1883) and was more recently studied by Wieland (1915). The base has the structure indicated by (I).



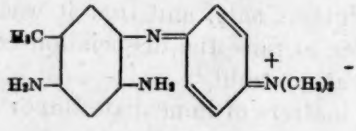
(I)

There will be occasion to compare the system, Bindschedler's green-leuco product, with the system methylene blue-leuco methylene blue, since the natures of the basic groups are the same in each case. Methylene blue base may be considered to be represented by (II).

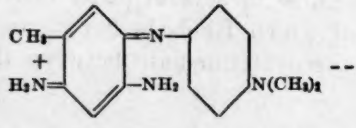


(II)

Toluylene blue (structure of base indicated by III*a* or III*b*) was first prepared by Witt (1879) by the interaction of



(III*a*)



(III*b*)

nitrosodimethyl-aniline hydrochloride and m-toluylenediamine. It may also be obtained by oxidation of the latter base together with dimethylparaphenylene diamine. The higher tinctorial power of toluylene blue will probably make it more useful as an indicator than Bindschedler's green if other considerations prove equal.

Toluylene blue in aqueous solution is transformed to the eurhodine neutral red (toluylene red) when heated. Undoubtedly, the transformation may proceed to some slight degree at room temperature. This sort of instability and a peculiar instability of Bindschedler's green, which will be discussed later, will make caution necessary in the use of these indamines.

However, the limitations can best be determined when there are provided the fairly reliable values of the characteristic constants which we have determined. Proceeding by the customary methods, we first determined the E_o' value of each sytem at some constant value of pH by titration of the oxidant (or the reductant) in a buffer solution. We then carried a fixed ratio of reductant to oxidant through a wide range of pH to determine the variation of E_o' with pH. The equation summarizing the general form of the relations found is, as might be expected, practically the same as the full equation for the methylene blue system (see Paper VIII). The inflexions of the E_o' : pH curve yield values for the several dissociation constants, some of which are confirmed by hydrogen electrode or by spectrophotometric measurements.

The reader who does not care to pursue the details will find summarized in Tables 17 and 18, and in the text of pages 20 to 24, the data required in practical applications.

In the course of determining the characteristic constants, we introduce a novel method for the proximate determination of endpoints, record certain spectrophotometric data, and deal briefly with the nature of and the rates of decomposition of Bindschedler's green.

Experimental

PREPARATIONS

Preparation of zinc chloride double salt of Bindschedler's green.— Seventeen and seventy-five hundredths grams p-aminodimethylaniline hydrochloride ($\frac{1}{10}$ mole) and 12.1 grams dimethylaniline were dissolved in 400 c. c. water to which had been added 15 c. c. concentrated hydrochloric acid. The solution was cooled to 0° C. It was then oxidized with a solution of 19.6 grams potassium dichromate (4 equivalents of oxygen) in 200 grams water to which had been added 5 c. c. glacial acetic acid. The oxidizing solution was added slowly and the reaction mixture well stirred. It was then heated to 50° C. and a solution of 25 grams zinc chloride in 50 c. c.

water was added. On standing, the solution deposited coppery, glistening crystals of the zinc chloride double salt of the dye. It was filtered, washed with 100 c. c. water, then with ethanol and ether. It was air-dried for one day, and then for two days over sulphuric acid. Yield = 32 g. = 89.6 per cent of theory.

Analysis: 0.2000 g. yielded 0.0226 g. ZnO.
0.2000 g. yielded 0.0228 g. ZnO.
Calc. for $(C_{16}H_{20}N_3Cl)_2ZnCl_2$: Zn, 9.13 per cent.
Found: Zn, 9.08, 9.16 per cent.

Preparation of leuco base of Bindschedler's green.—Thirty-two grams of the zinc chloride double salt of Bindschedler's green were dissolved in water and a current of hydrogen sulphide was passed into the solution until the green color disappeared. This solution was heated to boiling, filtered, and made alkaline with sodium hydroxide solution. This was extracted with ether, the ether removed by distillation, and the residue repeatedly crystallized from boiling gasoline until a product having a constant melting point was obtained. The pure product was colorless and was obtained in the form of glistening plates. M. P. 121° C. (corrected). Yield: 10 g., or 43.8 per cent of the calculated yield.

Analysis: 0.0826 g. yielded 12.2 c. c. N_2 (759 mm., 25° C.).
Calc. for $C_{16}H_{21}N_3$: N, 16.46 per cent.
Found: 16.37 per cent N.

The above was sample A. A second sample, B, gave the same melting point.

Preparation of toluylene blue.—Nitrosodimethylaniline hydrochloride was prepared according to the method described in Vanino's "Präparative Chemie" (1923). Thirty-six grams of this were dissolved in 500 c. c. hot water and the solution was cooled to 30° C. To this was added, with continuous stirring, a solution of 24 g. m-toluylene diamine in 500 c. c. water. The mixture was allowed to stand at room temperature for several hours. From it the dye separated as glistening blue crystals. Yield: 80 per cent of theory.

A sample dried at 80° C. under 12 mm. pressure for 24 hours was analyzed for nitrogen by the absolute method.

Substance: 0.1095 g., yielded 17.2 c. c. N_2 (763 mm., 25° C.).
Calculated for $C_{15}H_{19}N_4Cl + H_2O$: 18.14 per cent N.
Found: 18.07 per cent N.

POTENTIAL MEASUREMENTS

The buffer solutions used were those described by Table 4 of Paper VIII in this series. Since hydrogen electrode measurements were made in every instance, no attempt at high precision in the *construction* of these solutions was made.

As usual, the standard of electrode potential to which all values in this paper are reduced was that of a hydrogen electrode in $\frac{M}{20}$ KH-phthalate (−0.2386 volts).

All measurements were made at 30° C.

The apparatus used has been described in previous papers of this series.

In titrations of leuco Bindschedler's green with chromate, the end points were never sharp. Whereas potentials up to points near the end point were fairly steady, drifts appeared near the end point and became so serious that no definite approach of the titration curve to an asymptote was revealed. Accordingly, the following new method for the proximate estimation of the end point was employed as an aid.

Assuming a constant value for $[H^+]$, the equation relating potential, E_h, to the ratio of oxidant and reductant is

$$E_h = E'_o + \frac{RT}{nF} ln \frac{[S_o]}{[S_r]} \quad \text{---------- (1)}$$

If α is the degree of oxidation,

$$E_h = E'_o + \frac{RT}{nF} ln \frac{\alpha}{1-\alpha} \quad \text{---------- (2)}$$

Then

$$\frac{dE_h}{d\alpha} = \frac{RT}{nF} \frac{1}{(1-\alpha)\alpha} \quad \text{---------- (3)}$$

When $\alpha = 0.5$,

$$\frac{dE_h}{d\alpha} = \frac{4\ RT}{nF} \quad \text{---------- (4)}$$

When $n = 2$ and the temperature is 30° C.,

$$\frac{dE_h}{d\alpha} = 0.05222 \quad \text{---------- (5)}$$

This tangent to the titration curve is therefore a straight line defined by the conditions that it must pass through $E_h = E'_o$ when $\alpha = 0.5$ and must have a slope

$$\frac{\Delta E_h}{\Delta \alpha} = 0.05222$$

Its equation is, therefore,

$$E_h = E'_o - 0.02611 + 0.05222\,\alpha \quad \text{---------- (6)}$$

It is illustrated in Figure 1.
When $\alpha = 0$, ${}_0E_h = E'_0 - 0.02611$
When $\alpha = 1$, ${}_1E_h = E'_0 + 0.02611$
Then ${}_1E_h - {}_0E_h = 0.05222$.

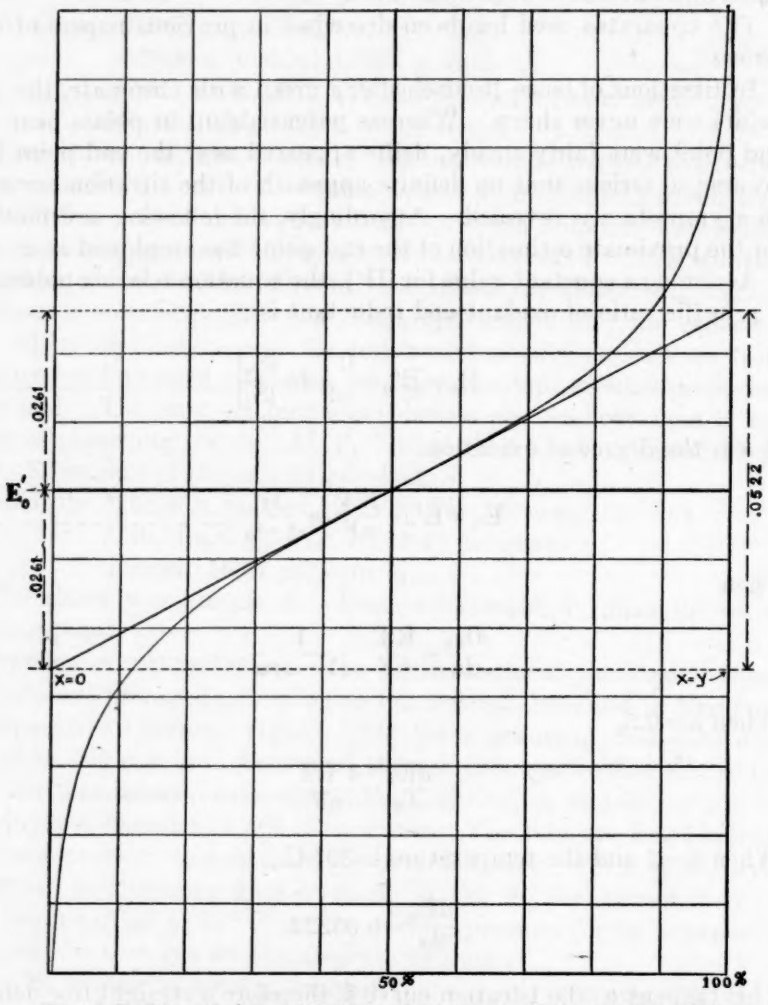


FIG. 1.—Graphic method for determining end-point in an oxidation-reduction titration involving a two-electron transfer

This furnishes tne means for determining the end point graphically. The experimental titration curve is plotted with E_h as ordinates and x centimeters of added oxidant as abscissas. The tangent is drawn to the mid-point of the curve and intersecting the ordinates at $x = 0$

and $x=y$ (where y is the end point sought). This tangent is defined by the equation

$$E_h = E'_o - 0.02611 + 0.05222\frac{x}{y} \qquad (7)$$

$$\left(\frac{x}{y}=\alpha\right)$$

The end-point y will be on this tangent at a point 0.02611 volts more positive than the mid-point, or 0.05222 volts more positive than the intersection of the tangent with the ordinate at $x=0$. The titration curve is ordinarily sufficiently "flat" near the mid-point to permit a fairly accurate placement of the tangent.

The usefulness of this procedure depends upon two matters of considerable importance. First, the measurements used to determine the middle section of the actual titration curve must be very accurate if the tangent is to be well placed. Second, there must not be superimposed upon the conditions defined by equation (1) a change of potential of different source such as would occur were the pH value of the solution to shift because of the formation of acid or base from the products of the reaction or because of dilution of the buffer. When such an effect tilts the tangent from its normal slope, there will be evident an appreciable discrepancy between the end point estimated by this graphic method and the end point estimated by the increase in increments of potential with fixed increments of oxidizing agent. In some of the present instances such discrepancies have amounted to 2 or 3 per cent in the end point, but we are not able definitely to ascribe this to changing pH, because titrations with chromate require considerable time and during this time there undoubtedly occurred slight but definite drifts of potential. We have, therefore, made more or less arbitrary selections of end points which inevitably smooth out the calculated values of E'_o, and in some of the tables give what may be a false impression of constancy. However, the constants so obtained are probably correct within 1 millivolt.

Incidentally it may be remarked that equation (3) gives the reciprocal of the poising effect, an analogy with the reciprocal of Van Slyke's (1922) buffer index in acid-base systems.

POTENTIAL MEASUREMENTS WITH THE BINDSCHEDLER'S GREEN SYSTEM

Electrode potential differences of solutions containing definite mixtures of Bindschedler's green and its leuco base were found to be fairly stable in buffer solutions of pH 2 or higher pH until the region of neutrality was approached.

The drifts of potential with time, distinctly noticeable but not serious in more acid solutions, began to become fast and finally rendered difficult or impossible the estimation of initial potentials in

solutions having pH values higher than 9.5. Throughout the region of pH higher than 2 the potential-change was always toward more negative values; while in solutions of pH value less than 2 the potential drift was positive.

In almost all cases, wherein the potential change was slow enough to obtain what was virtually a simultaneous measurement with each of the two electrodes always used, the potentials at these electrodes agreed within the limits of the precision of measurement (± 0.02 millivolt).

Since changes of potential were least in solutions between pH 2 and pH 5, the effort was made to obtain the orienting data in this region. Fairly representative of the drifts of potential in this region are the following: 0.02 millivolts per minute in solution No. 6; 0.03 millivolts per minute in solution No. 7; and 0.11 millivolts per minute in solution No. 8. Obviously such drifts, while not very serious, may introduce significant errors during titrations which occupy considerable time. It was, therefore, considered impracticable to introduce corrections for such factors as the change of pH which occurs during the titration by reason of the change in acid-base equilibria.

It had been observed (as recorded in paper VIII) that the potential of a fixed mixture of methylene blue and methylene white varied considerably with dilution. By analogy, a similar effect was to be expected with the Bindschedler's green system. Accordingly, a fixed mixture approximately 0.00125 molar with respect to oxidant and 0.00125 molar with respect to reductant was prepared in buffer No. 8 (pH 4.337). The data shown in Table 9, experiment No. 1 are not indicative of a significant effect of dilution, for the changes of potential found are not greater than the experimental error. A similar experiment in which an unbuffered mixture was added to buffer No. 7 also revealed no change greater than the experimental error.

The leuco base of methylene blue is very sensitive to light (see paper No. VIII). A solution of the dihydrochloride of Bindschedler's green left in a glass reservoir under nitrogen and exposed to the light of a room with northern exposure, showed no coloration during 10 days of observation.

Since the two preparations of the leuco base gave every evidence of being pure, orienting data were obtained by the titration of either sample of the leuco base. As will be shown, the potentials of the system are too positive for the employment of ferricyanide or quinone as oxidizing agents. Chlorine water acted as if it not only oxidized the leuco base to Bindschedler's green, but also produced an additional effect. This was evident in a slight, but appreciable, skew to the titration curve. Accordingly, K_2CrO_4 was used. This was dissolved in a portion of the same buffer solution that was used to stabilize the pH of the titrated base.

In Tables 1 to 4 are shown a series of such titrations. There has been excluded from detailed consideration a titration in buffer No. 6 with chlorine water which, by graphic analysis, gave a value of 0.471 for E'_0. (Compare with 0.4703 in Table 3.) As already noted, the evidence of a secondary action of chlorine was plain. There has also been excluded from consideration the first titration made, which was with ferricyanide in buffer 9. It was evident, and was later proved, that the potentials of the Bindschedler's green system and of the ferro-ferricyanide system overlap. However, the E'_0 value 0.346 to 0.347, estimated by rough graphic analysis, is close to the value calculated and given in Table 8.

In Tables 5, 6, and 7 are data obtained when fixed mixtures of oxidant and reductant were introduced into different buffer solutions, the pH value of the resulting mixture being considered equal to that of the buffer diluted to the same extent with water.

In the first instance a solution of the dihydrochloride of the leuco base was partially oxidized (while in the reservoir of the apparatus described by Figure 3 of Paper III), by drawing in a dilute solution of K_2CrO_4. In the second instance the oxidation was accomplished in the same manner, but with dilute chlorine-water. In spite of very slow addition and violent shaking, there was evident at points of contact with the chlorine-water a temporary color-change indicative of slight over-oxidation, perhaps irreversible in nature. In the third instance a mixture was prepared from the leuco base and the zinc double salt of the oxidant. By weights and volumes used, this should have been an equimolecular mixture. For a reason to be given later we suspect this was not an equimolecular mixture. Therefore, all three series of data need correction to obtain E'_0 values.

Comparison of the data for E_h in buffers No. 5 and No. 7 in Table 5 with the E'_0 values of Tables 1, 2, and 4 indicates corrections of 5.1, 5.7, and 6.0 millivolts. We shall use a weighted value of 5.8 millivolts, giving the series of E'_0 values of Table 5.

In Table 6 the E_h values for solutions Nos. 5, 6, and 7 differ from the E'_0 values of Tables 1, 2, 3, and 4 by 2.0, 2.6, 2.2, and 2.5 millivolts. We shall consider 2.3 millivolts the correction to be applied to the E'_0 values of Table 6.

The correction applied in Table 7 depends on later discussion.

Average values for E'_0 are assembled in Table 8.

In Table 9 are assembled a series of measurements with what should have been, by weights and volumes used, definite mixtures of oxidant and reductant. However, it was discovered that a solution of the zinc double salt of Bindschedler's green (sample B) in the absence of known reductant gave a potential which, while not entirely stable with respect to time, was exactly duplicated by each of two electrodes at any moment and which evidently lay in the region of

partial reduction. The percentage reduction was calculated to be 6.9 in one case and 7.9 in the other, these calculations being based on our final values of E'_0.

Now if the data be treated as if the oxidant and reductant were present in the proportions indicated by the volumes and concentrations of the solutions used, the average value for E'_0 is 0.3858, which is 2.5 millivolts below the value 0.3883 calculated from fairly reliable data. This divergence is probably much larger than the experimental error. Furthermore, the E'_0 values have a trend suggestive of disparity in the equivalence of the reductant and oxidant. Assuming, then, that reductant is present in solution B (from which A was prepared by mixture) to the extent of 7.4 per cent, the ratio of oxidant to reductant in solution B becomes $\frac{232}{19}$, and in solution A, $\frac{116}{134}$. Using these ratios and the volumes of A and B indicated in Table 9, there are found the "corrected" E'_0 values, which average 0.3882, only 0.1 millivolt below the calculated.

The experiment was repeated with entirely new solutions of A, B, and buffer No. 8. Again B alone gave potentials indicating the presence of the reductant, and A again gave a potential 2.4 millivolts below the calculated. This experiment happened to be incidental to another, and the aspect under present consideration was not in mind. Hence the detail is not so extensive; but calculations show that a reasonable correction will bring the data to within less than 0.5 millivolt of the calculated value of E'_0.

Since such corrections in their quantitative aspect are more or less arbitrary, no weight is given to the *corrected* E'_0 value of Table 9. However, since some correction is reasonable, no weight is given to the uncorrected value of E'_0 in the same table. The considerations just mentioned, when supported by an argument to be presented later, suggest the caution that must be used in the interpretation of data obtained by the method of mixtures.

In Figure 2 there are shown by the centers of circles the E'_0 values of the Bindschedler's green system as they are distributed on or near the E'_0: pH curve calculated from the equation presently to be discussed. For comparison there are shown the E'_0: pH curves of the Lauth's violet system and the methylene blue system described in the eighth paper of this series.

For a reason presently to be made clear, it was of particular interest to see whether the hydrogen electrode could be employed to follow the "titration curve" of the leuco base of Bindschedler's green and thereby to determine the first and second dissociation constants.

By reason of the low true solubility of the free base, it was necessary to operate with very dilute solutions in order to avoid precipitation of the free base. Accordingly, 0.1276 gm. of the leuco base was

dissolved under hydrogen in 200 c. c. of solution containing 6.6 c. c. 0.2012 normal HCl. Five c. c. of this solution were added to 50 c. c. de-aerated water contained in vessel A, Figure 1, of the third paper, this series. Contact with the saturated KCl-solution had previously

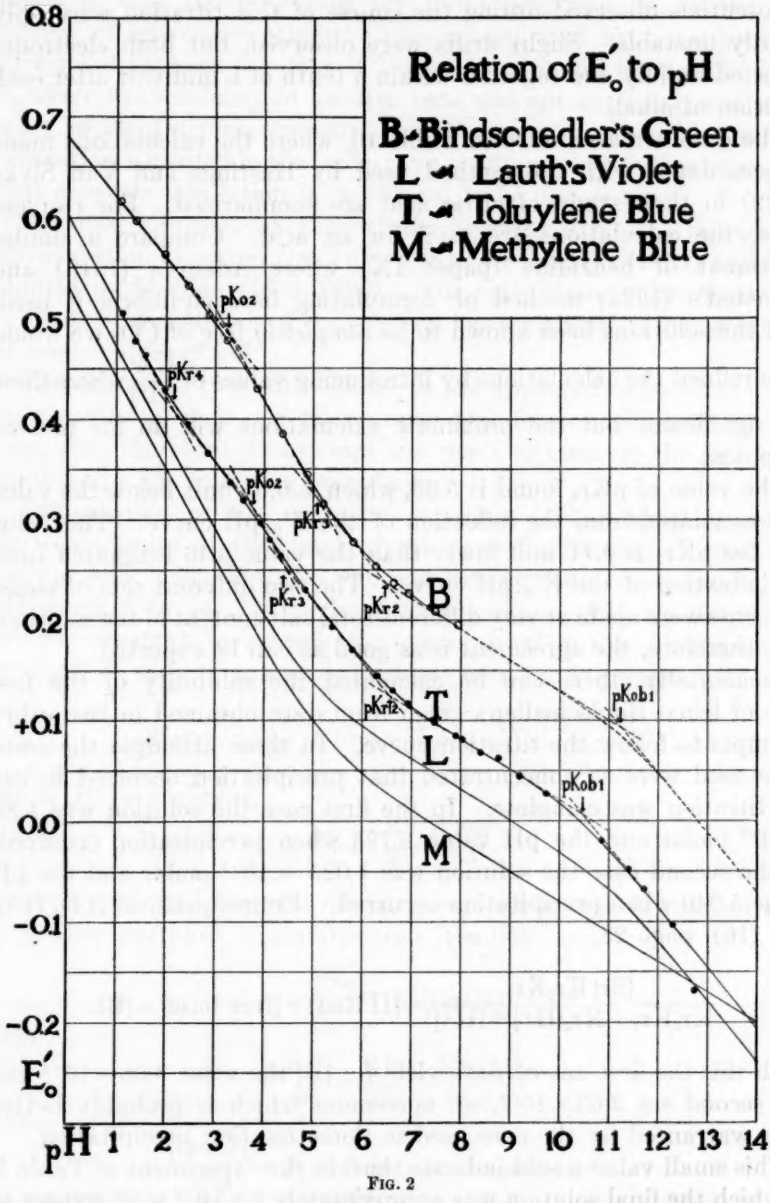


FIG. 2

been made with 5×10^{-3} N HCl, followed by rinsing of the vessel. When hydrogen had bubbled through the solution until the palladium-coated electrodes agreed and gave steady potentials, the solution was titrated with 1.001×10^{-3} N NaOH.

Since fair measurements were considered sufficient for present purposes, no *extreme* care was taken to make the alkali and the water used entirely free from CO_2—a matter of considerable importance for high accuracy with dilutions such as those used.

Potentials observed during the course of this titration were only slightly unstable. Slight drifts were observed, but both electrodes adjusted rapidly and together within a tenth of a millivolt after each addition of alkali.

The data are assembled in Table 10, where the calculations made in accordance with the method used by Hastings and Van Slyke (1922) in their study of citric acid are summarized. For convenience, the calculations are as if for an acid. Compare a similar treatment of benzidine (paper IX) where Adams's (1916) and Brønsted's (1923) method of formulating base equilibria is used. Had the solutions been known to be *completely* free of CO_2 we would have refined the calculations by introducing values of $\frac{K_w}{[H^+]}$ where these are significant, but the proximate calculations will do for present purposes.

The value of pKr_3 found is 5.05, which is 0.05 unit below the value 5.10 estimated from the inflection of the E'_0:pH curve. The value 6.35 for pKr_2 is 0.11 unit lower than the value 6.46 estimated from the inflextion of the E'_0:pH curve. The two different sets of measurements were made at very different total salt content of the solutions and, therefore, the agreement is as good as can be expected.

Incidentally, there can be calculated the solubility of the free base of leuco Bindschedler's green from data obtained in two other attempts to follow the titration curve. In these attempts the solutions used were so concentrated that precipitation occurred before the titration was complete. In the first case the solution was 1.89×10^{-3} molar and the pH value 5.723 when percipitation occurred. In the second case the solution was 1.025×10^{-3} molar and the pH value 5.940 when precipitation occurred. From equations (14), (15), and (16), page 23,

$$\frac{[Sr]\,Kr_2Kr_3}{Kr_2Kr_3 + Kr_3[H^+] + [H^+]^2} = [H\,Red] = [free\ base] = [B].$$

With this the first set of data yield for [B] the value 3.08×10^{-4} and the second set 2.63×10^{-4}, an agreement which is probably better than warranted by the care used in detecting first precipitation.

This small value would indicate that, in the experiment of Table 5, in which the final solution was approximately 5×10^{-4} with respect to the reductant, precipitation should have occurred at about 6.3.

Actually a dim clouding of solution No. 9 (pH 4.87) was noticed and a definite precipitate was observed in No. 10 (pH 5.4). This

indicates less true solubility in the buffer used. It would also seem to vitiate the measurements at the higher pH values. However, we have already noted the necessity for rapid measurements at the higher pH values, and, since this forced the use of initial potentials, it is probable that the potentials are those occurring before the slow process of separation sets in. This is confirmed by the substantial agreement between the data of Tables 5 and 6 and the data of Table 7, where the solubility of the free base was not exceeded, and where some incidental measurements, not shown, with one-fifth the concentration indicated in the table, gave results so close to those recorded that separate tabulation is considered to be unnecessary.

Alkaline solutions of Bindschedler's green exhibit a remarkable phenomenon, observed first, we believe, by Nietzki (1883) and later studied by Möhlau (1883–1885). The color changes irreversibly to brilliant blue, suddenly if the solution has a high pH value, gradually at lower values of pH.

The change in rate with change in pH appeared to be what would be expected were it dependent upon the decomposition of the free base, and, therefore, an attempt was made to measure the ionization constant by the following procedures.

Examination of the complete equation, (17) (page 24), and of the magnitudes of $[H^+]$ and of the several dissociation constants known, will show that the equation can be reduced to (a) for the pH range 8 to 14. In deriving this equation, $[S_r]$ has been considered constant to express the postulate that the decomposition products of the oxidant shall not react with the reductant.

$$E_h = C + \frac{RT}{2F} ln\,[So] - \frac{RT}{2F} ln\,\frac{Ko_{b1}[H^+] + K_w}{[H^+]^2} \quad \text{(a)}$$

When $[H^+]$ is constant,

$$dE_h = \frac{RT}{2F}\frac{d[So]}{[So]}$$

Assume now that the rate of disappearance of [So] is proportional to the concentration of the free base [OxOH]

$$\frac{-d[So]}{dt} = K_1[OxOH]$$

Then

$$\frac{-dE_h}{dt} = \frac{RT}{2F}\frac{K_1[OxOH]}{[So]}$$

But

$$\frac{[OxOH]}{[S_o]} = \beta, \text{ the degree of association.}$$

Hence

$$\frac{-dE_h}{dt} = \frac{RTK_1\beta}{2F} = K_2\beta \quad \text{(b)}$$

That equation (b) applies so far as its form is concerned is evident in the linear relations, shown in Figure 3, between E_h and t when β is held constant by a constant value of pH.

But

$$\beta = \frac{K_w}{K_w + Ko_{bl}[H^+]}$$

Hence

$$\frac{-dE_h}{dt} = \frac{K_2 K_w}{K_w + Ko_{bl}[H^+]} \text{----------(c)}$$

When $Ko_{bl}[H^+]$ is large in relation to K_w,

$$\frac{-dE_h}{dt} = \frac{K_2 K_w}{Ko_{bl}[H^+]}$$

or

$$\log \frac{-dE_h}{dt} - pH = \log \frac{K_2 K_w}{Ko_{bl}} = \text{a constant} \text{----------(d)}$$

That this relation holds with crude approximation for the cases in which the buffer was borate is evident in Table 11. The different order of magnitude of the value in the phosphate cases *suggests* a specific "salt effect." The fact that the value does not change greatly even at pH 9.7 indicates that Ko_{bl} is probably greater than 1×10^{-4}. If so, the measurements are in a region of pH, where β is small, and any error will produce a large effect on the value of Ko_{bl}. Hence only the last two sets of measurements (where β is probably *comparatively* large) will be used in the calculation of Ko_{bl} by equation (c).

For the case of buffer No. 23,

$$7.55 \times 10^{-4} = \frac{K_2 K_w}{1.88 \times 10^{-14} + Ko_{bl}(6.05 \times 10^{-10})} \text{---------(e)}$$

For the case of buffer No. 24,

$$23.4 \times 10^{-4} = \frac{K_2 K_w}{1.88 \times 10^{-14} + Ko_{bl}(1.93 \times 10^{-10})} \text{---------(f)}$$

Division of (f) by (e) and solution for Ko_{bl} yields $Ko_{bl} = 5.7 \times 10^{-3}$.

The precarious nature of such an attempt to measure Ko_{bl} is illustrated by the fact that if the pH value of buffer 24 were increased by 0.005 unit while the other data remained the same, the value of Ko_{bl}, then found would be 2.7×10^{-3}. Such an error in pH would correspond to a potential error of 0.3 millivolt in *some* potential measurement. Now a bold extrapolation of the value −12 for $\log \left(\frac{-dE_h}{dt}\right) - pH$ to comparatively low values of pH indicates that no drift of potential, due to the decomposition of the free base

and appreciable within the time of an ordinary experiment, should be observed in solutions near pH 3 or 4. Yet definite drifts were observed. This suggests that some factor other than the one under present discussion may have superimposed its effect at higher pH values. If so, the method of estimating $pK_{o_{bl}}$ is very precarious.

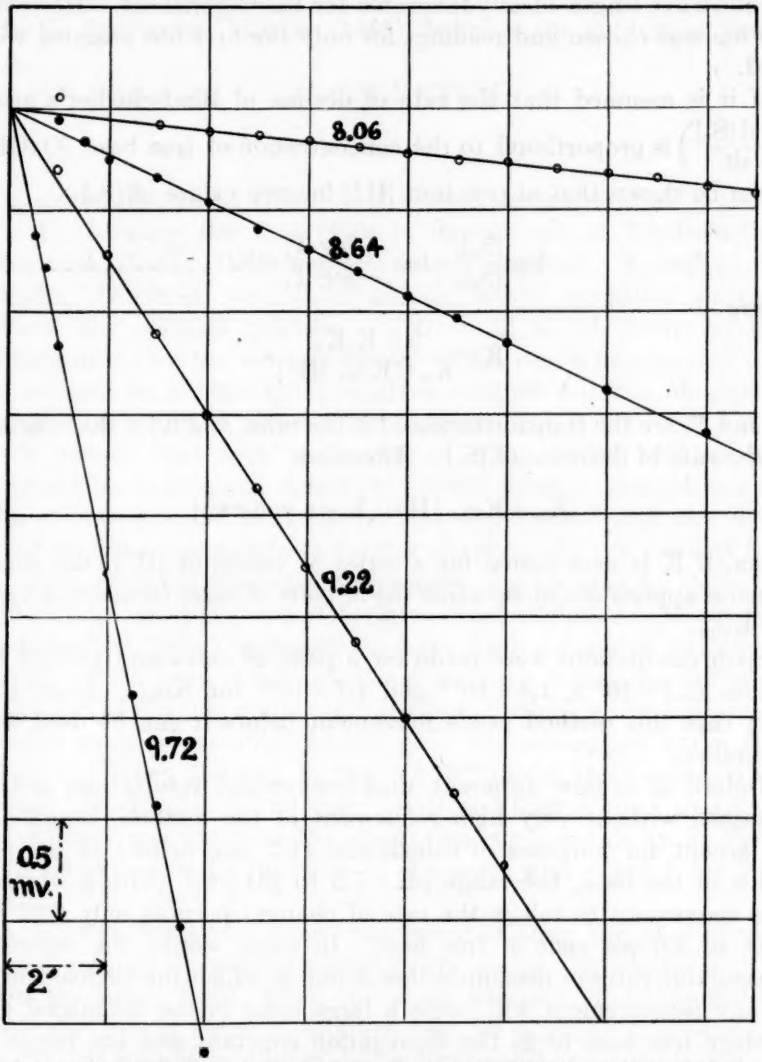


FIG. 3.—Bindschedler's green. Linear change of potential (ordinates) with time (abscissa) at indicated pH values due to decomposition of the free base of the oxidant

In an attempt to check this method, determinations were made of the increase in the light transmission at a given wave length with increase in the time during which solutions of Bindschedler's green were held at certain pH values. The absorption curves of Bindschedler's green, before and after treatment with alkali, were first

determined. In Figure 4, B is the absorption curve of Bindschedler's green at pH 7.5; A is the curve of the same solution after treatment with alkali; and C is the curve of the same solution after treatment for 20 hours at pH 8.1. In the latter case the readings were made with a 3 cm. tube, instead of a 10 cm. tube. It is evident that there is no one wave length ideally favorable for the experiment. However, 570 mμ was chosen and readings for only the first few minutes were used.

If it is assumed that the rate of decline of Bindschedler's green $\left(\frac{-d[S_o]}{dt}\right)$ is proportional to the concentration of free base, [OxOH], it can be shown that at constant $[H^+]$ for two values of $[S_o]$

$$\log \frac{[S_o]_1}{[S_o]_2} = \log \frac{-\log T_1}{-\log T_2} = Kt \text{----------} (g)$$

where

$$K = \frac{2.3\ K_1 K_w}{K_w + Ko_{bl}\ [H^+]},$$

T_1 and T_2 are the transmittancies, t is the time, and K_1 is the constant of the rate of decrease of $[S_o]$. Therefore

$$(K_w + Ko_{bl}\ [H^+])\ K = \text{a constant} \text{----------} (h)$$

Then, if K is determined for a series of values of $[H^+]$, the simultaneous application of equation (h) in pairs of cases furnishes a value for Ko_{bl}.

Such calculations were made for 3 pairs of cases and yielded the values (3.1×10^{-4}), 1.4×10^{-3} and 1.7×10^{-3} for Ko_{bl}. Again it is seen that this method needs refinement before it can be used with assurance.

Indeed it is now apparent that concordant results can not be obtained without very high refinement of the method, because, if we accept for purposes of calculation 11.2 (see below) as the pK_a value of the base, the range pH = 7.5 to pH = 9.7 (within which it was convenient to follow the rate of change) permits only 0.02 per cent to 3.0 per cent of free base. In other words, the region of measurable rates of decomposition is one in which the slightest error in any measurement will cause a large error in the estimated percentage free base or in the dissociation constant and the region of high percentage free base is one in which the rate of decomposition is too fast for ordinary measurement. The last statement is confirmed by the fact that in solution estimated to be sufficiently alkaline to set free practically all the base, the decomposition appears to be instantaneous. Therefore, we can do hardly more than guess that the inflexion of the E'_o:pH curve should occur at about pH 11.2 corresponding to pKo_{bl} 2.5 ($pK_w = 13.7$) or $Ko_{bl} = 3 \times 10^{-3}$, the latter

value being the average of the electrometric estimate and the more concordant of the values given above.

Aside from yielding a rather dubious approximation to a questionable dissociation constant, the experimental work on the alkaline solutions yielded some interesting facts concerning the nature of the products of decomposition.

In his first paper, Möhlau (1883) describes the product as phenol blue.

$$N\begin{cases}C_6H_4N(CH_3)_2\\ C_6H_4=O\end{cases}$$

In a subsequent paper (1885) he describes the preparation of phenol blue by treating the zinc chloride double salt of Bindschedler's green with alkali. In this process there occurred a flocculent precipitate. Dextrose was then added, which doubtless partially reduced the alkaline solution. It is to be noted, however, that Möhlau describes the solution as red. This would be expected were the solution to contain indophenol—a product which is obtained if the solution is heated without the dextrose.

We believe that with the excess of alkali which Möhlau used he would have obtained indophenol if the solution treated had not been so concentrated that phenol blue precipitated and was therefore partially protected from further change. As a matter of fact, we made repeated attempts to prepare phenol blue by Möhlau's method and found that the yields were extremely poor. Most of the Bindschedler's green appeared to have been converted into indophenol. With very dilute solutions, we have found the following: A solution of the zinc double salt of Bindschedler's green, sufficiently alkaline to cause a rapid change, gave the absorption curve B of Figure 4. When 500 c. c. of the 5×10^{-4} molar stock solution was treated with 25 c. c. 0.2 normal NaOH and then diluted to 5×10^{-6} molar, there was obtained the absorption curve shown by A, Figure 4. This is practically identical in character with curve I for indophenol.

A solution somewhat more concentrated than the above, when treated with alkali, yielded a strong odor of dimethylamine.

On the other hand, when the stock solution of Bindschedler's green was left 20 hours in mixture with an equal volume of buffer No. 20 (pH 8.1), then diluted to 1×10^{-5} molar and read in a 3 cm. tube, it gave curve C. This is practically identical with P, the curve of phenol blue. When the phenol blue solution was treated with alkali, it gave curve D, indicating a rather more radical change than that expected, but nevertheless suggesting some formation of indophenol.

Furthermore the Bindschedler's green, after treatment with alkali, gave a solution which turned red in neutral solution like indophenol. The pK_a value, determined by the method of Salm, was identical with that of indophenol (8.1) (see paper V this series).

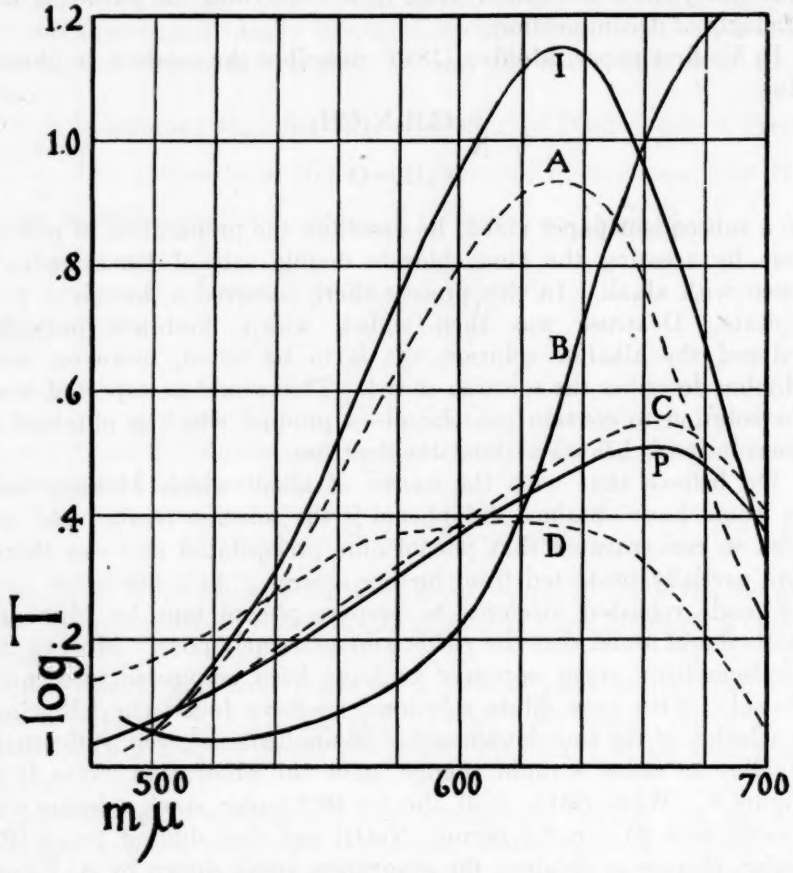


FIG. 4.—Spectrophotometric curves of Bindschedler's green and some of its decomposition products

B = Bindschedler's green 5×10^{-6} molar at pH 7.5 in 10 cm. tube

A = Bindschedler's green after treatment with dilute alkali and diluted to 5×10^{-6} molar at pH 12.3 in 10 cm. tube

I = Indophenol 5×10^{-6} molar at pH 12.3 in 10 cm. tube

C = Bindschedler's green 20 hours at pH 8.1 diluted to 1×10^{-5} molar in 3 cm. tube

P = Phenol blue 1×10^{-6} molar at pH 8.1 in 3 cm. tube

D = Phenol blue after treatment with dilute alkali. Diluted to 1×10^{-5} molar in 3 cm. tube

The Bindschedler's green solution which had been held at pH 8.1 for 20 hours behaved like phenol blue in its changes of color with change of pH.

Phenol blue does not go to indophenol so readily as does Bindschedler's green.

ELECTRODE MEASUREMENTS WITH TOLUYLENE BLUE

Electrode potentials in mixtures of toluylene blue and its leuco base were much like those observed in the Bindschedler's green system in that they were fairly stable between pH 2.5 and 9.0. They were much less stable than the potentials of the Bindschedler's green system in the acid solutions and very much more stable in the alkaline solutions. However, a new uncertainty enters. Toluylene blue is easily transformed to neutral red. Preliminary measurements of the neutral red system (*cf.* Clark & Zoller, 1921) have shown that the latter has a characteristic potential very negative to that of the toluylene blue system so that there should be no interference, and the effect of neutral red production should be exclusively in its alteration of the ratio of toluylene blue to leuco-toluylene blue. This effect we have not studied. Rather we have sought to obtain known ratios by cold reduction and fairly rapid manipulation of solutions.

In Tables 12, 13, and 14 are shown titration data. In Table 15, Series II, measurements were made upon a solution divided into two parts, one of which was reduced with hydrogen and platinized asbestos, filtered, and stored under nitrogen.

The measurements of E_h in Series I and Series III were made with two different solutions of toluylene blue partially reduced with hydrogen and platinized asbestos. In Series I the values of E_h in buffers 4, 5, and 15 were respectively 0.0103, 0.0093, and 0.0112—average 0.0103 volts negative to the values of E'_0 found in Tables 12, 13, and 14. (The divergences of E_h from the average correspond to errors of 0.0, 1.4, and 1.7 per cent reduction.) Accordingly, the E_h values in Series I are corrected by $+0.0103$ to obtain the E'_0 values.

In Series III, Table 15, the E_h values observed in the region of pH where potentials are fairly stable, averaged 0.0010 volt more negative than corresponding values of E'_0 in Series I and II. Hence this value was used uniformly to find E'_0 for Series III.

The E'_0 values calculated by means of equation (18), page 24, are shown in the next to the last column of Table 15. In Figure 2 there are charted the average values of E'_0, and the curve determined by equation 18 with the values of the several constants listed in Table 15.

An attempt was made to determine the titration curve of leuco toluylene blue by means of hydrogen electrode measurements. The potentials were nearly as satisfactory as those obtained with leuco Bindschedler's green and clearly indicated at least two basic groups with dissociation constants near those calculated by the oxidation-reduction measurements. However, no very consistent values for the constants were obtained, chiefly because it was necessary to work with dilutions so high that the correction for the hydrions coming

from the hydrochloric acid predominate in attempts to evaluate the second basic constant. The calculations were therefore abandoned when nothing inconsistent with the results of other measurements was detected.

SPECTROPHOTOMETRIC DETERMINATION OF DISSOCIATION CONSTANTS FOR OXIDANT OF TOLUYLENE BLUE

The transmittancies were determined with a Keuffel and Esser color analyzer, tube length, 10 cm. Concentrations of dye in different buffers were varied from 2×10^{-6} to 5×10^{-5} molar. In all cases, 50 c. c. buffer were diluted with 5 c. c. dye solution, and the pH of the buffers was determined in a dilution of 50:5 with distilled water.

From the values of $-\log T$ at the wave length 650 mμ there were calculated the pK values of the dissociations in the acid and alkaline regions. In the acid region buffers yielded transformations of 10.8, 26.3, and 53.8 per cent at pH values of 2.862, 3.316, and 3.819, respectively. These data yield the values 3.779, 3.764, and 3.753, respectively—average, 3.765. With percentage transformations less than 10 per cent, the values were considerably divergent and were discarded. No determinations were made at transformations above 53.8 per cent.

In the alkaline region, transformations of 17.5, 41.9, and 79.3 per cent at pH values of 11.40, 10.86, and 9.695, respectively, gave the pK values 10.73, 10.72, and 10.28, respectively—average, 10.58. Transformations above 79.3 per cent gave divergent results which were discarded.

There is reason to believe that the color undergoes change with time, both in the acid and alkaline regions, so that the above values for the dissociation constants are probably only approximations.

The value $pK_w - pKo_{bl}$ determined spectrophotometrically at about 20° is 10.58, while the value which fits the electrometric data at 30° is 10.48. The value of pKo_2 determined spectrophotometrically at about 20° is 3.77. We have used the rounded value 3.8 in the calculation of E'_o values.

COMPARISON OF INDAMINES WITH THIAZINES

In Table 16 are assembled the *basic* dissociation constants determined electrometrically for oxidant and reductant of toluylene blue, Bindschedler's green, Lauth's violet, and methylene blue.

Comparison of the E'_o:pH curves, shown in Figure 2, reveals a certain parallelism between the curves for the four systems, two of which are described in the 8th paper of this series.

The structural formulae are also comparable; and so far as the participation of ionization is concerned, the equations for each

system (excluding that for toluylene blue) should be the same—with different values, of course, for E_o and for the several ionization constants. In the discussion of the methylene blue and the Lauth's violet systems, we made the following remark:

> Since the electrometric data reveal *directly* little regarding the nature or location of the acid-base groups encountered, it is possible to express the experimental data by a number of equations derived in accordance with the principles outlined in the second paper of this series. Without claiming to have exhausted the possibilities, we have constructed several such equations which express the experimental data well enough, but which call for bizarre chemical properties in the thiazines. But by adopting the following rational development, we have reached a result which seems satisfactory from every viewpoint.

We need not repeat the argument; it was a question of identifying the ionization constants as determined by the inflexions of the E'_o : pH curve with definite allocations to specific groups in the oxidant and in the reductant. The inflexion of each E'_o : pH curve from a ".03-slope" to a ".09-slope" was ascribed to ionization of the two basic groups of the reductant, the lack of a distinctive intermediate ".06-slope" being ascribed to the small difference in the values of pKr_2 and pKr_3. A similar situation is seen in the Bindschedler's green curve, and in this instance we now have the values of Table 10 directly determined by hydrogen electrode measurements.

This leaves no doubt that other inflexions must be ascribed to the basic groups of the oxidant provided we retain the very reasonable assumption, discussed in Paper VIII, that the absence of any tendency toward a "0-slope" of the E'_o : pH curve indicates that the central nitrogen binds firmly a hydrion.

Comparing the oxidants we find the following interesting facts. If the polar group should have a *basic* dissociation constant weak enough, there will occur an inflexion at measurable values of pH. Decomposition of Bindschedler's green prevents the direct detection of this inflexion. The methylene blue system shows none. On the other hand, both the Lauth's violet and the toluylene blue systems show this inflexion. If the amino group has a dissociation constant high enough, there will occur an inflexion of the E'_o : pH curve from ".09-slope" to ".06-slope" in acid solutions. None was surely detected in either Lauth's violet or methylene blue, although suggested by one measurement in 5 N HCl. But this inflexion is now distinct in the Bindschedler's green curve; and it is also present in the toluylene blue curve where it is produced by one of the amino groups of the oxidant. That it can be identified in each case with an ionization of the oxidant is evident not only in the direction of the inflexion but also in the color change which, when applied in Salm's method, gives a dissociation constant practically identical with that determined by the center of inflexion of the E'_o : pH curve.

Thus if the four systems are considered together there is left no doubt whatever of the correctness of the formulation given in Paper VIII.

There remains the interesting question of whether the dominant form of toluylene blue is represented by formula III *a* or by III *b* (page 2). There is also the possibility of an orthoquinone structure analogous to III *b*. The fact that one basic dissociation constant of toluylene blue is of the high order of magnitude of one constant for each oxidant of the other systems merely indicates a polar group.

There are three basic dissociation constants (corresponding to two amino groups and one dimethylamino group) to be allocated in the oxidant of toluylene blue. The fact that the toluylene blue curve in acid regions shows no break from the ".09-slope," indicates that one of these basic dissociations (Ko_{b3}) is too weak to be detected and can be reasonably assigned to one of the amino groups.

It is also to be expected that a dimethylamino group in a compound of this nature will have a basic dissociation greater than that of a simple amino group. It follows, therefore, that the second basic dissociation (Ko_{b2}) in the oxidant of toluylene blue must be assigned to the remaining simple amino group, and that the largest dissociation constant (Ko_{b1}) belongs to the dimethylamino group which is presumably polar. If this reasoning is valid, structure III *a* for toluylene blue may be regarded as the more probable.

EQUATIONS

In the treatment of analogous cases described in paper VIII, the equation was abbreviated by leaving out of consideration the undetected ionizations. The more complete equation would have been (8)

$$E_h = E_o - \frac{RT}{nF} ln \frac{[Sr]}{[S_o]}$$

$$- \frac{RT}{nF} ln \frac{Ko_{b1}Ko_2[H^+] + Ko_2K_w + Ko_{b1}[H^+]^2}{Kr_1Kr_2Kr_3[H^+] + Kr_2Kr_3[H^+]^2 + Kr_3[H^+]^3 + [H^+]^4} \quad \text{---} \quad (8)$$

which is derived from equations (9) to (16), the constants being defined by (9), (10), (13), (14), and (15).

$$\frac{[\overset{+}{Ox}]\,[\overset{-}{OH}]}{[OxOH]} = Ko_{b1} \text{ (dissociation of polar group of oxidant)} \quad \text{---------} \quad (9)$$

$$\frac{[\overset{+}{Ox}]\,[H^+]}{[\overset{++}{OxH}]} = Ko_2 \text{ (equivalent to association of } H^+ \text{ at amino group of oxidant)} \quad \text{----------} \quad (10)$$

$$[S_o] = [OxOH] + [\overset{+}{Ox}] + [\overset{++}{OxH}] \quad \text{----------} \quad (11)$$

$$[\bar{OH}]\,[H^+] = K_w = 1.88 \times 10^{-14} \text{ at } 30° \quad (12)$$

$$\overset{+}{Ox} + 2e \rightleftarrows \bar{Red}$$

$$\frac{[\bar{Red}]\,[H^+]}{[H\ Red]} = Kr_1 \text{ (binding of hydrion at bridging N of reductant)} \quad (13)$$

$$\frac{[H\ Red]\,[H^+]}{[H_2{}^+Red]} = Kr_2 \text{ (equivalent to association of } H^+ \text{ at first amino group of reductant)} \quad (14)$$

$$\frac{[H_2{}^+Red]\,[H^+]}{[H_3\overset{++}{Red}]} = Kr_3 \text{ (equivalent to association of } H^+ \text{ at 2d amino group of reductant)} \quad (15)$$

$$[Sr] = [\bar{Red}] + [H\ Red] + [H_2{}^+Red] + [H_3\overset{++}{Red}] \quad (16)$$

Since Kr_1 is negligibly small, the product in (8) where it occurs is negligible in the sum.

We have estimated for the Bindschedler's green system the values for Kr_2, Kr_3, and Ko_2 by the methods outlined in paper VIII and have used an arbitrary value for Ko_{b1}, all of which are indicated in Table 8. By the use of $pK_w = 13.73$ (30°) there are calculated the *basic* dissociation constants which are shown in Table 16 and which can there be compared with the values for components of the Lauth's violet, methylene blue, and toluylene blue systems.

In this table we have uniformly used the values determined by the oxidation-reduction experiments. The hydrogen electrode measurements of the constants of the reductant were made at a different total salt content and these display substantial agreement with the results of the other type of measurement.

In formulating the system toluylene blue-leuco toluylene blue, consideration must be given to an additional basic group in the reductant and an additional basic group in the oxidant. The latter was not detected, either because it is too weak to detect, or because measurements at high acidity are very uncertain. Consequently it will tentatively be neglected and the numerator of the last term of the equation remains as it is in (8). Consideration of the extra basic group in the reductant

$$\frac{[H^+]\,[H_3\overset{++}{Red}]}{[H_4\overset{+++}{Red}]} = Kr_4$$

makes the denominator of the last term

$$Kr_1Kr_2Kr_3Kr_4[H^+] + Kr_2Kr_3Kr_4[H^+]^2 + Kr_3Kr_4[H^+]^3 + Kr_4[H^+]^4 + [H^+]^5$$

but again Kr_1 is negligibly small. Hence for toluylene blue the equation is (18) and for Bindschedler's green the equation is (17).

$$E_h = E_o - \frac{RT}{2F} ln \frac{[Sr]}{[So]} - \frac{RT}{2F} ln \frac{Ko_{b1}Ko_2[H^+] + Ko_2K_w + Ko_{b1}[H^+]^2}{Kr_2Kr_3[H^+]^2 + Kr_3[H^+]^3 + [H^+]^4} \quad (17)$$

$$E_h = E_o - \frac{RT}{2F} ln \frac{[Sr]}{[So]} - \frac{RT}{2F} ln \frac{Ko_{b1}Ko_2[H^+] + Ko_2K_w + Ko_{b1}[H^+]^2}{Kr_2Kr_3Kr_4[H^+]^2 + Kr_3Kr_4[H^+]^3 + Kr_4[H^+]^4 + [H^+]^5} \quad (18)$$

Values for E_h when $\frac{[Sr]}{[So]} = 1$ (E'_o values) calculated by means of these equations are shown in Tables 8 and 15.

CONDENSED TABLES OF POTENTIALS

To save the reader the laborious task of calculating potentials from percentage color change and pH, there are assembled in Table 17 the E'_o values for several indicators (including Bindschedler's green and toluylene blue) at intervals of 0.2 pH.

In all cases the relation

$$E_h = E'_o - 0.03006 \log \frac{[Sr]}{[So]}$$

applies at constant pH and at 30° C. The relations between percentage reduction and $-0.03006 \log \frac{[Sr]}{[So]}$ are shown in Table 18.

If, for example, there is observed a 90 per cent reduction of toluylene blue in a solution known to have a pH value of 6.6 the calculation of the oxidation-reduction potential is as follows: Table 17 shows that toluylene blue at pH 6.6 and 50 per cent reduction should indicate at 30° C. a potential of +0.132: Table 18 shows that at 90 per cent reduction the potential is 0.029 volt more negative. Hence 0.132 − 0.029 = +0.103. For ordinary purposes, linear interpolations will probably be adequate for intermediate pH values, or intermediate percentages reduction.

While the data are for 30°, standardization of calculations in terms of these data can be used until temperature coefficients are determined. Descriptions of solutions to which the indicators are applied should be stated, for it will undoubtedly be necessary later to develop systematic investigations of several possible sources of error.

Summary and Conclusions

By the procedures here set forth, the principles of which are described in previous papers of the series, there have been determined in buffers of various pH values the electrode potentials of mixtures of Bindschedler's green and its leuco base and of mixtures of toluylene blue and its leuco base. All potentiometric measurements were at 30° C.

For the Bindschedler's green system the rationally developed equation found to apply is

$$E_h = E_o - \frac{RT}{2F} ln \frac{[Sr]}{[So]} - \frac{RT}{2F} ln \frac{Ko_{b1} Ko_2 [H^+] + Ko_2 K_w + Ko_{b1} [H^+]^2}{Kr_2 Kr_3 [H^+]^2 + Kr_3 [H^+]^3 + [H^+]^4}$$

where E_h is the potential difference between electrode and solution, E_o is a charactertistic constant, [So] and [Sr] are the concentrations of total Bindschedler's green and of the leuco base, respectively, and the several dissociation constants are defined as follows:

$\frac{[Ox^+]\,[\bar{O}H]}{[OxOH]} = Ko_{b1}$ = dissociation constant of polar group of oxidant;

$\frac{[Ox^+]\,[H^+]}{[\overset{+}{Ox}\overset{+}{H}]} = Ko_2$ = dissociation of hydrion from dimethyl amino group;

$\frac{[H\ Red]\,[H^+]}{[H_2\overset{+}{Red}]} = Kr_2$ = dissociation of hydrion from one dimethyl amino group of reductant;

$\frac{[H_2\overset{+}{Red}]\,[H^+]}{[H_3\overset{+}{\overset{+}{Red}}]} = Kr_3$ = dissociation of hydrion from the other dimethyl amino group of reductant.

One hydrogen is supposed to enter combination with the bridging nitrogen in the reductant.

Ko_{b1} was only roughly estimated to be about 3×10^{-8}. This estimate was based on the influence of pH on the rate of decomposition of Bindschedler's green in alkaline solution, when, in agreement with previous work, it was shown that the setting free of the base results in the replacement of the polar dimethyl amino group by oxygen to form phenol blue, followed further by replacement of the other group to form indophenol.

From inflexions of the E'_o:pH curve the other constants were evaluated as follows:

$Ko_2 = 5.4 \times 10^{-4}$, or corresponding basic constant 3.47×10^{-11}

$Kr_2 = 3.5 \times 10^{-7}$, or corresponding basic constant 5.37×10^{-8}

$Kr_3 = 8.0 \times 10^{-6}$, or corresponding basic constant 2.35×10^{-9}

At pH = 0 the potential is +0.680

The values of Kr_2 and Kr_3 were confirmed by hydrogen electrode measurements of the titration curve.

Potentials are fairly stable only between pH 2 and pH 9.5. The oxidant is subject to decomposition. Its tinctorial power is low. It will, therefore, be serviceable as an indicator only for preliminary purposes. This is the first indamine to be characterized potentiometrically, and it suggests that the potentials of indamines will be in nearly the same general region as those of the indophenols.

The equation for toluylene blue is

$$E_h = E_o - \frac{RT}{2F} ln \frac{[S_r]}{[S_o]} - \frac{RT}{2F} ln \frac{Ko_{b1}Ko_2[H^+] + Ko_2Kw + Ko_{b1}[H^+]^2}{Kr_2Kr_3Kr_4[H^+]^2 + Kr_3Kr_4[H^+]^3 + Kr_4[H^+]^4 + [H^+]^5}$$

The several symbols are defined in a manner analogous to the above, but by reason of an additional amino group the number of dissociation constants increases.

Evaluated by inflexions of the E'_o:pH curve, the values of the dissociation constants are

$Ko_{b1} = 5.62 \times 10^{-4}$

$Ko_2 = 1.58 \times 10^{-4}$ $\quad \frac{Kw}{Ko_2} = 1.19 \times 10^{-10}$ (by spectrometric measurement)

$Kr_2 = 2.73 \times 10^{-7}$ $\quad \frac{Kw}{Kr_2} = 6.83 \times 10^{-8}$

$Kr_3 = 4.03 \times 10^{-5}$ $\quad \frac{Kw}{Kr_3} = 4.72 \times 10^{-10}$

$Kr_4 = 7.26 \times 10^{-3}$ $\quad \frac{Kw}{Kr_4} = 2.60 \times 10^{-12}$

At pH = 0 the potential of the 50 per cent reduced solution is +0.601. The order of magnitude of the dissociation constants of the reductant was confirmed by hydrogen electrode measurements, but the high dilution required prevented accurate computations.

The values of $pKw - pKo_{b1}$ determined spectrophotometrically at 20° is 10.58 (10.48 at 30° by electrometric method), and of $pKw - pKo_2 = 3.77$ (3.8 used in calculations).

Comparison of the data for the indamines with the data for the structurally similar thiazines reveals the essential correctness in the allocation of dissociation constants.

A new method for graphic estimation of the end-point of oxidation-reduction titration is given. It involves a treatment of poising index comparable to Van Slyke's buffer index.

The two indicators which have been characterized are suggested as possible substitutes for comparable indophenols but with the caution that their basic nature and their instability may introduce difficulties when the indicators are applied to biochemical problems.

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TABLE 1.—*Bindschedler's green*

Titration of 5 c. c. approximately 0.01 molar leuco Bindschedler's green dihydrochloride (A) in 50 c. c. buffer No. 5 with K_2CrO_4 in buffer No. 5.

[pH of 50 c. c. buffer No. 5+5 c. c. water=2.862. Time required, 1 hour]

Chromate	Oxidation	$0.03006 \log \frac{[S_r]}{[S_o]}$	E_h	E'_o
C. c.	*Per cent*			
2	8	+0.0319	0.4723	0.5042
4	16	+.0216	.4825	.5041
6	24	+.0151	.4890	.5040
8	32	+.0098	.4940	.5038
10	40	+.0053	.4984	.5037
12	48	+.0010	.5025	.5036
14	56	−.0031	.5067	.5036
16	64	−.0075	.5109	.5034
18	72	−.0123	.5155	.5032
20	80	−.0181	.5209	.5028
22	88	−.0260	.5280	(.5020)
25	100		.5552	
Average				.5036

TABLE 2.—*Bindschedler's green*

Titration of 5 c. c. approximately 0.01 molar leuco Bindschedler's green dihydrochloride (A) (a slight excess of HCl) in 50 c. c. buffer No. 5 with K_2CrO_4 in buffer No. 5.

[pH of 50 c. c. buffer No. 5+5 c. c. water=2.862. Time required, 1 hour]

Chromate	Oxidation	$0.03006 \log \frac{[S_r]}{[S_o]}$	E_h	E'_o
C. c.	*Per cent*			
1	4.50	0.0399	0.4637	0.5036
2	9.01	.0302	.4738	.5040
3	13.51	.0243	.4799	.5042
4	18.02	.0198	.4845	.5043
5	22.52	.0161	.4882	.5043
6	27.03	.0130	.4914	.5044
7	31.53	.0101	.4940	.5041
8	36.04	.0075	.4966	.5041
9	40.54	.0050	.4991	.5041
10	45.05	.0026	.5016	.5042
11	49.55	+.0002	.5040	.5042
12	54.06	−.0021	.5062	.5041
14	63.06	−.0070	.5112	.5042
16	72.08	−.0124	.5168	.5044
18	81.08	−.0190	.5237	.5047
20	90.09	−.0288	.5349	(.5061)
21	94.60	−.0374	.5450	(.5076)
22.2	100.00			
Average				.5042

TABLE 3.—*Bindschedler's green*

Titration of 5 c. c. approximately 0.01 molar leuco Bindschedler's green dihydrochloride (A) in 50 c. c. buffer No. 6 with K_2CrO_4 in buffer No. 6 diluted (50-5).

[pH of 50 c. c. buffer No. 6+5c. c. water=3.327. Time, 1.2 hours]

Chromate	Oxidation	$0.03006 \log \frac{[Sr]}{[So]}$	E_h	E'_o
C. c.	*Per cent*			
1	4.39	+0.0402	0.4290	(0.4692)
2	8.77	+.0306	.4394	(.4700)
3	13.16	+.0246	.4456	.4702
4	17.54	+.0202	.4501	.4703
6	26.32	+.0134	.4569	.4703
8	35.09	+.0080	.4623	.4703
10	43.86	+.0032	.4671	.4703
12	52.63	−.0014	.4717	.4703
14	61.40	−.0061	.4764	.4703
16	70.18	−.0112	.4815	.4703
18	78.95	−.0173	.4877	.4704
20	87.72	−.0257	.4960	.4703
22	96.49	−.0434	.5117	(.4683)
22.8	100.00			
Average				.4703

TABLE 4.—*Bindschedler's green*

Titration of 5 c. c. approximately 0.01 molar leuco Bindschedler's green dihydrochloride (A) (slight excess HCl)+50 c. c. buffer No. 7 with K_2CrO_4 in buffer No. 7.

[pH of 50 c. c. buffer No. 7+5 c. c. water=3.838. Time, 1.5 hours]

Chromate	Oxidation	$0.03006 \log \frac{[Sr]}{[So]}$	E_h	E'_o
C. c.	*Per cent*			
1	5.38	0.0374	0.3908	(0.4282)
3	16.13	.0215	.4079	.4294
5	26.88	.0131	.4167	.4298
7	37.63	.0066	.4232	.4298
9	48.39	+.0008	.4289	.4297
11	59.14	−.0048	.4346	.4298
13	69.90	−.0110	.4408	.4298
15	80.65	−.0186	.4484	.4298
17	91.40	−.0309	.4604	.4295
18	96.78	−.0445	.4725	(.4280)
18.6	100.00			
Average				.4297

TABLE 5.—*Bindschedler's green—Relation of E'_o to pH*

Series 1: 50 c. c. 0.01 molar leuco Bindschedler's green partially oxidized with approximately 5 c. c. 0.1N chromate solution—5 c. c. of this added to 50 c. c. various buffers.

Buffer No.	pH	E_h	E'_o
1	1.077	0.6121	0.6179
3	1.977	.5565	.5623
5	2.862	.4985	.5043
7	3.838	.4237	.4295
9	4.872	.3396	.3454
10	5.399	.2993	.3051
13	6.275	.2480	.2538
15	6.942	.2210	.2268
17	7.956	.1899	.1957

TABLE 6.—*Bindschedler's green—Relation of E'_o to pH*

Series 2: 75 c. c. 0.01 molar Bindschedler's green+about 25 c. c. 0.03 N chlorine water—5 c. c. of this added to 50 c. c. buffers.

Buffer No.	pH	E_h	E'_o
1	1.077	0.6107	0.6130
2	1.374	.5952	.5975
4	2.439	.5299	.5322
5	2.862	.5016	.5039
6	3.327	.4681	.4704
7	3.838	.4272	.4295
8	4.364	.3842	.3865
9	4.872	.3429	.3452
10	5.399	.3040	.3063
11	5.812	.2773	.2796
13	6.275	.2517	.2540
14	6.618	.2354	.2377
15	6.942	.2231	.2254
16	7.494	.2057	.2080
17	7.956	.1946	.1969

TABLE 7.—*Bindschedler's green—Relation of E'_o to pH*

Series 3: 1 c. c. or 5 c. c. of a solution containing 0.0025 molar dihydrochloride of leuco base (sample B) and 0.0025 molar Bindschedler's green zinc double salt (sample B), added to 50 c. c. of each buffer

Buffer No.	pH 50:5 dilution	Amount of solution used	E'_o	E'_o corrected [1]
		C. c.		
1	1.077	5	0.6141	0.6160
3	1.977	5	.5600	.5619
5	2.862	5	.5027	.5046
7	3.838	1	.4282	.4301
8	4.350	5	.3848	.3867
9	4.872	5	.3440	.3459
11	5.812	5	.2775	.2794
14	6.618	5	.2366	.2385
16	7.494	1	.2053	.2072

[1] See Table 9 and text.

TABLE 8.—*Bindschedler's green—Average values of E'₀*

Buffer No.	pH	E'₀	E'₀ calculated [1]	Deviation
1	1.077	0.6157	0.6153	+0.0004
2	1.374	.5975	.5974	+.0001
3	1.977	.5622	.5607	+.0015
4	2.439	.5322	.5318	+.0004
5	2.862	.5043	.5039	+.0004
6	3.327	.4704	.4704	.0000
7	3.838	.4298	.4299	−.0001
8a	4.350	.3868	.3872	−.0004
8	4.364	.3865	.3861	+.0004
9	4.872	.3455	.3450	+.0005
10	5.399	.3057	.3065	−.0008
11	5.812	.2796	.2803	−.0007
13	6.275	.2539	.2551	−.0012
14	6.618	.2382	.2392	−.0010
15	6.942	.2261	.2262	−.0001
16	7.494	.2087	.2071	+.0016
17	7.956	.1963	.1924	+.0039

[1] Calculated by means of equation (17) using

$$E'_{pH0} = E_0 - 0.03006 \log K_{ob1} = 0.6801$$
$$E_0 = 0.5742$$
$$K_{ob1} = 3\times10^{-3} \qquad pK_w - pK_{ob1} = 11.2$$
$$K_{ob2} = 5.4\times10^{-4} \qquad pK_{ob} = 3.27$$
$$K_w = 1.88\times10^{-14} \qquad pK_w = 13.73$$
$$K_{r3} = 8\times10^{-6} \qquad pK_{r3} = 5.10$$
$$K_{r2} = 3.5\times10^{-7} \qquad pK_{r2} = 6.46$$

TABLE 9.—*Mixtures of Bindschedler's green and its leuco product. Solution A: zinc double salt of Bindschedler's green (sample B), and dihydrochloride of leuco product (sample B) by weight 0.00125 molar oxidant and 0.00125 molar reductant in undiluted buffer No. 8. Solution B: 0.0025 molar oxidant in buffer No. 8. Solution B used in preparation of solution A*

[pH of undiluted buffer 8=4.337. E'₀ calculated from data and equation of Table 8=0.3883]

Experiment No.	Buffer No. 8	Solution B	Solution A	E_h	0.03006 log [Sr]/[So]	E'₀	Corrected 0.03006 log [Sr]/[So]	Corrected E'₀
	C. c.	*C. c.*	*C. c.*					
1	50	0	1	0.3858	0	0.3858	+0.0019	0.3877
			2	.3858	0	.3858	.0019	.3877
			6	.3860	0	.3860	.0019	.3879
			10	.3862	0	.3862	.0019	.3881
			20	[1] .3867–0.3855	0			
2	50	1	4	0.3914	−0.0053	0.3861	−0.0029	0.3885
			6	.3898	−.0038	.3860	−.0015	.3883
			8	.3890	−.0029	.3861	−.0008	.3882
			10	.3886	−.0024	.3862	−.0003	.3883
			15	.3881	±.0016	.3865	+.0004	.3885
			20	.3878	−.0012	.3866	+.0008	.3886
3	50	2	0	[2] 0.4222			−0.0330	0.3892
			4	.3945	−0.0091	0.3854	−.0062	.3883
			6	.3923	−.0067	.3856	−.0042	.3881
			8	.3911	−.0053	.3858	−.0029	.3882
			10	.3903	−.0044	.3859	−.0021	.3882
1	50	3	0	[3] 0.4203			−0.0330	0.3873
			2	.4017	−0.0181	0.3836	−.0137	.3880
			5	.3954	−.0103	.3851	−.0073	.3881
			10	.3919	−.0061	.3858	−.0037	.3882
			20	.3898	−.0034	.3864	−.0012	.3886
Average						.3858		.3882

[1] Drift in 8 minutes.
[2] Corresponds to about 6.9 per cent reductant.
[3] Corresponds to about 7.9 per cent reductant.

TABLE 10.—*Titration of dihydrochloride of leuco Bindschedler's green with NaOH. Dissociation constants calculated as if for a diacidic acid*

$[NaOH]=1.0106\times10^{-3}$ N

A = c. c. 0.001 normal NaOH − equivalent of excess HCl

V = volume of solution in c. c.

$C=\frac{0.0025\times5}{V}$ = molar conc. of leuco Bindschedler's green

$Br=\frac{0.001\times A}{V}$

$B'_1=Br-B'_2$

$B''_2=Br-B''_1$

$B'_2=\frac{C(4.5\times10^{-7})}{[H^+]+(4.5\times10^{-7})}$

$B''_1=\frac{C(8.9\times10^{-6})}{[H^+]+(8.9\times10^{-6})}$

$pK_1=pH+\log[C-(B'_1+[H^+])]-\log(B'_1+[H^+])$

$pK_2=pH+\log[C-(B''_2+[H^+])]-\log(B''_2+[H^+])$

NaOH	A	V	C×10⁴	Br×10⁴	B'₂×10⁴	B'₁×10⁴	pH	[H⁺]×10⁶	pK₁
C. c.	*C. c.*	*C. c.*							
0		55					4.215		
0.5							4.263		
1		56					4.314		
2	0.47	57	2.19	0.0825	0.254	0.067	4.416	3.84	(5.002)
3	1.48	58	2.16	.2552	.323	.223	4.529	2.96	5.029
4	2.49	59	2.12	.4220	.407	.381	4.639	2.30	5.032
5	3.50	60	2.08	.5833	.513	.532	4.749	1.78	5.034
6	4.51	61	2.05	.7393	.647	.675	4.860	1.38	5.042
7	5.52	62	2.02	.8903	.808	.810	4.965	1.08	5.044
8	6.54	63	1.98	1.038	.997	.938	5.071	.849	5.042
9	7.55	64	1.95	1.180	1.232	1.057	5.176	.667	5.043
10	8.56	65	1.92	1.315	1.532	1.162	5.285	.519	5.050
11	9.57	66	1.89	1.450	1.898	1.260	5.395	.403	5.052
12	10.58	67	1.87	1.579	2.344	1.344	5.503	.314	5.059
13	11.59	68	1.84	1.704	2.885	1.416	5.616	.242	5.060
14	12.60	69	1.81	1.826	3.496	1.476	5.725	.188	5.049
Average									5.05

					B''₁×10⁴	B''₂×10⁴			pK₂
15	13.61	70	1.79	1.944	1.54	4.04	5.838	0.145	6.354
16	14.62	71	1.76	2.059	1.56	4.99	5.948	.113	6.337
18	16.64	73	1.71	2.279	1.59	6.89	6.170	.0676	6.334
19	17.65	74	1.69	2.385	1.60	7.85	6.292	.0511	6.349
20	18.66	75	1.67	2.488	1.60	8.88	6.406	.0393	6.337
21	19.67	76	1.64	2.588	1.59	9.98	6.542	.0287	6.347
22	20.68	77	1.62	2.686	1.58	11.06	6.690	.0204	6.355
23	21.69	78	1.60	2.781	1.58	12.01	6.872	.0134	6.382
24	22.70	79	1.58	2.873	1.57	13.03	7.104	.0079	(6.430)
Average									6.35
24.5	23.21						7.26		
25	23.72						7.50		
25.4	24.12						7.80		
25.8	24.52						8.20		
26	24.73						8.36		
26.2	24.93						8.53		
26.5	25.23						8.69		

$K_1=Kr_1=\frac{K_w}{Kr_{b1}}$. Hence $pKr_1=5.05$; $Kr_{b1}=2.11\times10^{-9}$.

$K_2=Kr_2=\frac{K_w}{Kr_{b2}}$. Hence $pKr_2=6.35$; $Kr_{b2}=4.21\times10^{-8}$.

TABLE 11.—*Bindschedler's green*

Change of potential per minute in solutions of different pH value containing initially approximately 50% reduced Bindschedler's green 0.0001 molar. (30° C.)

Buffer No.	Nature of buffer	$\frac{-dE_h}{dt}$	$\log\left(\frac{-dE_h}{dt}\right)$	pH	$\log\left(\frac{-dE_h}{dt}\right)_{-pH}$
16	Na_2HPO_4+HCl	0.000024	−4.620	7.494	−12.114
17	Na_2HPO_4+HCl	.000083	−4.081	7.956	−12.037
20	$H_3BO_3+NaCl+NaOH$	.000058	−4.237	8.060	−12.297
22	$H_3BO_3+NaCl+NaOH$	.000226	−3.646	8.643	−12.289
23	$H_3BO_3+NaCl+NaOH$	.000755	−3.122	9.218	−12.340
24	$H_3BO_3+NaCl+NaOH$	.002340	−2.631	9.715	−12.346
25	$Na_2HPO_4+NaCl+NaOH$	(1)			

[1] Too high to measure.

TABLE 12.—*Toluylene blue*

Titration of 2.5×10^{-3} molar leuco toluylene blue with K_2CrO_4 in buffer No. 5. ($pH=2.862$.)

K_2CrO_4	Oxidation	$0.03006\log\frac{[S_r]}{[S_o]}$	E_h	E_o'
C. c.	*Per cent*			
2	12.27	0.0257	0.3397	0.3654
4	24.54	.0147	.3509	.3656
6	36.81	.0070	.3586	.3656
7	42.94	.0037	.3620	.3657
8	49.08	+.0005	.3653	.3658
9	55.21	−.0027	.3685	.3658
10	61.35	−.0060	.3718	.3658
11	67.49	−.0095	.3753	.3658
12	73.62	−.0134	.3792	.3658
13	79.76	−.0179	.3837	.3658
14	85.89	−.0236	.3892	.3656
15	92.03	−.0319	.3972	.3653
16	98.16	−.0518	.4134	(.3626)
16.3	100.00			
Average				.3657

TABLE 13.—*Toluylene blue*

Titration of 2.5×10^{-3} molar leuco toluylene blue with K_3FeCy_6 in buffer No. 15. ($pH=6.926$.)

K_3FeCy_6	Oxidation	$0.03006\log\frac{[S_r]}{[S_o]}$	E_h	E_o'
C. c.	*Per cent*			
0	0		0.045	
1	6.78	0.0342	.0845	0.1187
2	13.56	.0242	.0945	.1187
3	20.34	.0178	.1007	.1185
4	27.12	.0129	.1056	.1185
5	33.90	.0087	.1099	.1186
6	40.68	.0049	.1137	.1186
7	47.46	+.0013	.1173	.1186
8	54.24	−.0022	.1207	.1185
9	61.02	−.0058	.1244	.1186
10	67.80	−.0097	.1282	.1185
11	74.58	−.0140	.1325	.1185
12	81.36	−.0192	.1377	.1185
13	88.14	−.0262	.1447	.1185
14	94.92	−.0382	.1561	.1189
14.75	100.00			
Average				.1186

TABLE 14.—*Toluylene blue*

Titration of 2.5 × 10^{-3} molar leuco toluylene blue with K_2CrO_4 in buffer No. 4. (pH = 2.450.)

K_2CrO_4	Oxidation	$0.03006 \log \frac{[S_r]}{[S_o]}$	E_h	E'_o
C. c.	*Per cent*			
2	7.69	0.0324	0.3650	0.3974
4	15.38	.0222	.3731	.3953
6	23.08	.0157	.3791	.3948
8	30.77	.0106	.3838	.3944
10	38.46	.0061	.3881	.3942
12	46.15	+.0020	.3921	.3941
14	53.82	−.0020	.3961	.3941
16	61.54	−.0061	.4002	.3941
18	69.23	−.0106	.4047	.3941
20	76.92	−.0157	.4099	.3942
22	84.62	−.0222	.4165	.3943
24	92.31	−.0324	.4283	.3959
26	100.00			
Weighted value				.3941

TABLE 15.—*Toluylene blue—Relation of E'_o to pH*

Buffer No.[1]	pH	Series I		Series II	Series III		Average	Calculated	Deviation
		E_h	E'_o	E'_o	E_h	E'_o	E'_o	E'_o	
1	1.086	0.498	0.508	0.501	0.505	0.506	0.505	0.5042	+0.0008
2	1.365	.470	.480		.477	.478	.479	.4800	−.0010
2 1/2	1.580				.462	.463	.463	.4617	+.0013
3	1.978	.420	.430				.430	.4294	+.0006
4	2.450	.3838	*.3941*				.3941	.3942	−.0001
5	2.862	.3564	*.3657*	.3659	.3642	.3652	.3656	.3659	−.0003
6	3.316	.3251	.3354		.3351	.3361	.3358	.3356	+.0002
6 1/2	3.570				.3175	.3185	.3185	.3184	+.0001
7	3.819	.2917	.3020				.3020	.3011	+.0009
7 1/2	4.092				.2806	.2816	.2816	.2820	−.0004
8	4.336	.2539	.2642		.2634	.2644	.2643	.2650	−.0007
9	4.842	.2205	.2308	.2304			.2306	.2309	−.0003
10	5.380	.1850	.1953		.1945	.1955	.1954	.1972	−.0018
11	5.787	.1618	.1721				.1721	.1733	−.0012
13	6.262	.1363	.1466	.1470			.1468	.1478	−.0010
13	6.275				.1462	.1472	.1472	.1472	.0000
14	6.612	.1211	.1314		.1299	.1309	.1312	.1312	.0000
15	6.926	.1074	*.1186*				.1186	.1181	+.0005
15	6.946				.1162	.1172	.1172	.1173	−.0001
16	7.469	.0885	.0988				.0988	.0986	+.0002
17	7.903	.0743	.0846	.0847			.0847	.0846	+.0001
20	8.019	.0712	.1815				.0815	.0810	+.0005
21	8.403				.0678	.0688	.0688	.0692	−.0004
22	8.787	.0522	.0625	.0626			.0625	.0574	+.0051
23	9.207	.0340	.0443				.0443	.0444	−.0001
24	9.695	.0180	.0283	+.0280			+.0282	+.0284	−.0002
25	10.86			−.0188			−.0188	−.0207	+.0019
26	11.40			−.0488			−.0488	−.0501	+.0013
27	11.72			−.0666			−.0666	−.0686	+.0020
29	12.30			−.1029			−.1029	−.1029	.0000
30	12.75			−.1666			−.1666	−.1299	−.0367

[1] Solutions designated with "1/2" made from approximately equal parts of the adjacent solutions.

$pK_w - pK_{o_{b1}} = 10.48$ $\quad K_{o_{b1}} = 5.62 \times 10^{-4}$ $\quad pK_{r_2} = 6.56$ $\quad K_{r_2} = 2.73 \times 10^{-7}$

$pK_{o_2} = 3.8$ $\quad K_{o_2} = 1.58 \times 10^{-4}$ $\quad pK_{r_3} = 4.40$ $\quad K_{r_3} = 4.03 \times 10^{-5}$

$E'_{pH_0} = 0.6011$ $\quad pK_{r_4} = 2.14$ $\quad K_{r_4} = 7.26 \times 10^{-3}$

TABLE 16.—*Basic ionization constants, pH values of inflexions in E'_o: pH curves (corresponding to pKa values) and characteristic potentials at pH 0*

Basic groups	Symbol	Methylene blue		Lauth's violet		Bindschedler's green		Toluylene blue	
		Value of constant	Inflexion at pH	Value of constant	Inflexion at pH	Value of constant	Inflexion at pH	Value of constant	Inflexion at pH
Oxidant's polar	K_{Ob1}	Too high to measure	None.	1.88×10^{-3}	11.0	(3×10^{-3})?	(11.2)?	5.62×10^{-4}	10.48
Oxidant's first nonpolar	K_{Ob2} (K_{O2})[1]	Too low to measure	None.	Too low to measure	None.	3.47×10^{-11}	3.27	1.19×10^{-10}	3.80
Oxidant's second nonpolar	K_{Ob3} (K_{O3})[1]	None	None.	None	None.	None	None.	Too low to measure	None.
Reductant's bridging N	K_{Rb1} (K_{R1})[1]	Fixes H^+	None.	Fixes H^+	None.	Fixes H^+	None.	Fixes H^+	None.
Reductant's first basic	K_{Rb2} (K_{R2})[1]	1.35×10^{-6}	5.85	3.8×10^{-9}	5.30	5.37×10^{-8}	6.46	6.83×10^{-8}	6.56
Reductant's second basic	K_{Rb3} (K_{R3})[1]	6.3×10^{-10}	4.52	4.5×10^{-10}	4.38	2.35×10^{-9}	5.10	4.72×10^{-10}	4.40
Reductant's third basic	K_{Rb4} (K_{R4})[1]	None	None.	None	None.	None	None.	2.60×10^{-12}	2.14
Characteristic potential	E'_{pH0}	0.532		0.563		0.680		0.601	

[1] $K_{O2}=\frac{K_w}{K_{Ob2}}$, etc.

TABLE 17.—*E'_o values for several oxidation-reduction indicators. 30° C. (Values rounded to nearest millivolt)*

pH	Indigo disulphonate	Indigo trisulphonate	Indigo tetrasulphonate	Methylene blue	Toluylene blue	1-Naphthol-2-sulphonate indo, 3', 5' dichlorophenol	1-Naphthol-2-sulphonate indophenol	2, 6-Dichlorophenol indo o-cresol	2, 6-Dichlorophenol indophenol	Bindschedler's green	o-Chlorophenol indophenol	m-Bromophenol indophenol
5.0	−0.010	0.032	0.065	0.101	0.221	0.262	--------	0.335	0.366	0.335	--------	--------
5.2	−.022	.020	.053	.088	.208	.249	--------	.322	.352	.320	--------	--------
5.4	−.034	+.008	.041	.077	.196	.236	(1)	.307	.339	.307	(1)	(1)
5.6	−.045	−.004	.029	.066	.184	.223	--------	.292	.325	.293	--------	--------
5.8	−.057	−.016	.017	.056	.173	.210	--------	.277	.310	.281	--------	--------
6.0	−.069	−.028	+.006	.047	.162	.196	0.183	.261	.295	.270	0.301	--------
6.2	−.081	−.039	−.006	.039	.151	.181	.171	.245	.279	.259	.288	--------
6.4	−.092	−.051	−.017	.031	.141	.166	.159	.228	.263	.249	.275	(1)
6.6	−.104	−.061	−.027	.024	.132	.150	.147	.212	.247	.240	.262	--------
6.8	−.114	−.072	−.037	.017	.123	.134	.135	.196	.232	.232	.248	--------
7.0	−.125	−.081	−.046	.011	.115	.119	.123	.181	.217	.224	.233	0.248
7.2	−.134	−.091	−.055	+.004	.108	.103	.111	.166	.203	.217	.218	.235
7.4	−.143	−.099	−.062	−.002	.101	.088	.099	.152	.189	.210	.203	.221
7.6	−.152	−.107	−.070	−.008	.094	.073	.087	.138	.175	.204	.187	.208
7.8	−.160	−.114	−.077	−.014	.088	.060	.074	.125	.162	.197	.170	.193
8.0	−.167	−.121	−.083	−.020	.082	.046	.062	.112	.150	--------	.155	.178
8.2	−.174	−.127	−.090	−.026	.075	.034	.049	.099	.137	--------	.139	.163
8.4	−.180	−.134	−.096	−.032	.069	.021	.036	.087	.125	--------	.124	.148
8.6	−.187	−.140	−.102	−.038	.063	+.010	.023	.075	.113	(2)	.109	.133
8.8	−.193	−.146	−.108	−.044	.057	−.002	+.010	.063	.101	--------	.095	.117
9.0	−.199	−.152	−.114	−.050	.051	−.012	−.003	.051	.089	--------	.082	.103

[1] Unstable in this region of pH. [2] Decomposes in this region of pH.

TABLE 18.—*Relation of percentage reduction to potential at constant pH determined by*

$$E_h = E'_o - 0.03006 \log \frac{[Sr]}{[So]} \text{ at } 30° \text{ C.}$$

[Values rounded to nearest millivolt]

Reduction	$-0.03006 \log \frac{[Sr]}{[So]}$	Reduction	$-0.03006 \log \frac{[Sr]}{[So]}$
Per cent	*Volts*	*Per cent*	*Volts*
1	+0.060	55	−0.003
2	+.051	60	−.005
5	+.038	65	−.008
10	+.029	70	−.011
15	+.023	75	−.014
20	+.018	80	−.018
25	+.014	85	−.023
30	+.011	90	−.029
35	+.008	95	−.038
40	+.005	98	−.051
45	+.003	99	−.060
50	±.000		

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